

REMARKS

The above changes were made to correct typographical or other technical errors. No new matter has been added.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 300622004900. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

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EXHIBIT A. VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

On page 6:

The present invention provides recombinant host cells and expression vectors for making products in host cells, which are otherwise unable to make those products due to the lack of a biosynthetic pathway to produce a precursor required for biosynthesis of the product. As used herein, the term recombinant refers to a cell, compound, or composition produced at least in part by human intervention, particularly by modification of the genetic material of a cell. The present invention also provides methods for increasing the amounts of a product produced in a host cell by providing recombinant biosynthetic pathways for production of a precursor utilized in the biosynthesis of a product.

On page 56:

To express active mutase *in vivo*, it was necessary to grow cells in a defined media (MUT media) that allows uptake of the vitamin B12 precursor hydroxocobalamin; this is similar to an established protocol for expression of active methionine synthase, which also requires B12. Cell extracts overexpressing the mutase were shown to convert mm-CoA to succinyl CoA without the addition of vitamin B12. Only one time point (at 20 minutes) was assayed to confirm activity; the specific activity of the mutase [must] was not determined.

On page 56:

Thus, methylmalonyl-CoA mutase was expressed as the active holoenzyme in *E. coli*, and methylmalonyl-CoA was produced *in vivo*. Because a slow, spontaneous

chemical epimerization between (R)- and (S)-mm-CoA does exist (approximately 3% in 15 minutes), it may be [helpful] helpful to determine the relative amounts of these diastereomers in cells overexpressing the mutase. Enough (S)-mm-CoA may be present to support polyketide production in some cells without addition of an epimerase. To facilitate the eventual production of polyketides in *E. coli*, the mutase gene can be incorporated into the chromosome of the BL21 *panD* cell or other host cell.

On page 69:

Epimerase activity was measured in crude extracts of *E. coli* harboring either pET-epsherm, pET-epcoel, pET-epsub, or a control pET vector. The epimerase assay couples transcarboxylase, which converts (S)-methylmalonyl-CoA into propionyl-CoA, to malate dehydrogenase, which converts NADH into NAD⁺, producing a decrease in absorbance at 340 nm. The assay is initiated with a racemic mixture of (R,S)-methylmalonyl-CoA; when the (S)-isomer is consumed as described below[;], a steady background rate is observed at about one-tenth of the initial rate. When an extract containing epimerase is added to the assay, the (R)-isomer is converted to (S)-, resulting in a further decrease in absorbance. In crude *E. coli* extracts, however, a significant background rate is observed, probably due to an endogenous NADH oxidase. Thus the epimerase must be expressed at a sufficiently high level to conclude that it is active. The assay was conducted as follows.

On page 73:

Most modular PKSs require either or both malonyl-CoA or (2S)-methylmalonyl-CoA as a source of 2-carbon units for polyketide biosynthesis. The malonyl-CoA pools in yeast are quite sufficient for polyketide synthesis, as illustrated by the production of large amounts of 6-MSA in yeast. However, *S. cerevisiae* does not produce (2S)-methylmalonyl-CoA and does not possess biosynthetic pathways for methylmalonyl-CoA biosynthesis. Hence, a heterologous biosynthetic pathway must be introduced into

S. cerevisiae to support biosynthesis of polyketides that use (2*S*)-methylmalonyl-CoA as a precursor.

On page 82:

As described in Example 1, methylmalonyl-CoA epimerase was purified from *Propionibacterium shermanii* and N-terminal and internal protein sequence was obtained. Degenerate PCR primers based on the amino acid sequences were designed and were used to amplify a 180 bp PCR product from *P. shermanii* genomic DNA. The PCR product was labeled and used to isolate the epimerase gene from [a] *P. shermanii*. The methylmalonyl-CoA epimerase genes from *B. subtilis* [16] and *S. coelicolor* can also be employed in the methods of the present invention.

On page 83:

Propionyl-CoA is not detected in *E. coli* SJ16 cells grown in the presence of [³H] β -alanine with or without the addition of propionate in the growth media. When *E. coli* SJ16 cells were transformed with a pACYC-derived plasmid containing the *Salmonella typhimurium* propionyl-CoA ligase gene (*prpE*) under the control of the *lac* promoter, a small amount of propionyl-CoA was observed (~0.2% of total CoA pool) in cell extracts. When 5 mM sodium propionate was included in the culture medium, about 14-fold more propionyl-CoA was produced (~3% of the total CoA pool). [These results are shown graphically below.]